In Vivo Anti-diabetic and Anti-oxidant Potential of *Psoralea corylifolia* Seeds in Streptozotocin Induced Type-2 Diabetic Rats

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This study was made to investigate the anti-diabetic and antioxidant potential of ethanolic extract of seeds of *Psoralea corylifolia* L. in streptozotocin (STZ) nicotinamide induced type-2 diabetic rats. Male Albino Wistar rats (150–250 g) were taken for the study. The ethanolic extract of seeds of *Psoralea corylifolia* was prepared and the oral administration of this extract tested for anti-hyperglycemic activity in streptozotocin-nicotinamide induced diabetic rats showed significant effect on blood glucose level in acute and chronic study. The body weight, oral glucose tolerance test and biochemical parameter such as insulin level, liver glycogen contents, glycosylated hemoglobin, total plasma cholesterol and antioxidant parameters were estimated for all treated group and compared against diabetic control group. In oral glucose tolerance test both dose (200 and 400 mg/kg) intensively reduced external glucose level. After 28 days (chronic study) of treatment with extract the maximum reduction in blood glucose level were observed in streptozotocin-nicotinamide rats with ethanolic extract 400 mg/kg body weight per os. There were significant increase in body weight, liver glycogen content, plasma insulin level and decrease in the blood glucose, glycosylated hemoglobin level and total plasma cholesterol. However it also influences on the antioxidant parameters like decreases malondialdehyde (MDA) level and increases reduced glutathione (GSH) level. This investigation results that *Psoralea corylifolia* has significant anti-hyperglycemic and antioxidant activity.

Key words —— hyperglycaemia, streptozotocin, *Psoralea corylifolia*, glibenclamide

INTRODUCTION

Diabetes mellitus is one of the most common leading causes of death illness, a major global health and economic problem, characterized by high levels of blood glucose resulting from defects in insulin production, insulin action, or both. Diabetes is affecting 6% of the world's population and about 7% of the U.S.A. population.[1, 2] Those who has diabetes, type-2 is the most common type, accounting for 90–95% of all diabetic cases.[2] The 60–90% people who has diabetes are obese but not all when the disease is diagnosed.[3] Worldwide projections suggest that more than 300 million people will have diabetes by 2025 and the global cost of treating diabetes and its complications could reach 1 trillion U.S. dollar annually.[4] The disease is characterized by chronic hyperglycaemia as a relative or absolute lack of insulin, or the action of insulin on its target tissue, or both.[5] Both forms (type-1 and type-2) of diabetes are associated with major long-term complications, including cardiomyopathy, angiopathy, *etc*.

Psoralea corylifolia L. is a widely used medicinal plants commonly known as Baguchi in Sanskrit and Bavachi in Hindi, grown in Asia and India.[6, 7] The seeds are extensively used in variety of disease such as ringworm, leucoderma,[8, 9] premature ejaculation, treatment of impotence,[10] psoriasis, alopecia and vitiligo,[11] act as antitumor,[12] checking diarrohea,[13] shows antibacterial activity,[14, 15] antioxidant, stimulant, osteoblastic proliferation and also show vasodilatory effect.[16]

There are several type of phytochemical such as flavones, isoflavanoids, furanocoumarins and chal-

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cones derived from the seeds of *Psoralea corylifolia*. The reported main chemical constituents are psoralidin,[17] angelicin, psoralen, isobavachalone, neobavaisoflavone, bavachin, coumarins, backuchiol, daidzin and uracil. These all constituent possess a number of biological activities. The plant is used traditionally in the treatment of diabetes but not scientifically proved till date. So the work has planned to prove scientifically that this plant is used to combat this metabolic disorder for the welfare of the society.

MATERIALS AND METHODS

Drugs and Chemicals —— The drugs and chemicals that are used in the study includes glibenclamide (Torrent Pharmaceutical, Ahmadabad, India), streptozotocin (STZ), heparin (SRL, New Delhi, India), EDTA (Hi-media Lab. Pvt Ltd., Mumbai, India), Ellman's reagent [5,5′-dithiobis-(2-nitro-benzoic acid); DTNB], sodium sulphate, methanol, pyridine, anthrone, thiourea, benzoic acid, sodium chloride (SD Fine Chem Ltd., Mumbai, India). All the chemicals used in the study were of analytical grade.

Preparation of Plant Extract —— *Psoralea Corylifolia* seeds were purchased from local market of Hisar was identified and authenticated by Dr. H. B. Singh, Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (Ref. NISCAIR/RHMD/Consult/-2009-10/1310/113), Delhi (India). The seeds were dried at $40 \pm 1°C$, grounded into a granulated powder and defatted with petroleum ether. The ethanolic extract was obtained by extracting 3 kg of defatted seed powder with ethanol (95%) at 50°C for 72 hr in soxlet followed by filtration and concentrated in rotary vacuum evaporator at $50 \pm 5°C$. The percentage yield of the extract was found 2.92.

Experimental Animals —— Healthy male albino wistar rats (150–250 g, 60–90 days old) were procured from Disease Free Small Animal House, Chaudhary Charan Singh Haryana Agriculture University, Hisar (Haryana, India). The rats were housed in polycarbonate cage size ($29 \times 22 \times 14$ cm) under laboratory standard conditions ($25 \pm 3°C$: 35–60% humidity) with alternating light and dark cycle of 12 hr each and were feed fed with a standard rat pellet diet (Hindustan Lever Ltd, Mumbai, India) and water *ad libitum*. The experimental protocol

was approved by Institutional Animals Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India (Registration No. 0436).

Induction of Diabetes —— The animal model of type-2 diabetes mellitus (NIDDM) was induced in overnight fasted animals by a single intraperitoneal injection of 60 mg/kg STZ, 15 min after the intraperitoneal (i.p.) administration of 120 mg/kg nicotinamide. Hyperglycemia was confirmed by the elevated blood glucose levels determined at 72 hr then on 7th day of the injection. Only rats confirmed with permanent NIDDM were used in the anti-diabetic study.[18]

Experimental Design —— Rats were divided into five groups comprising six rats in each batch after the induction of diabetes. Group 1: normal control rats administered 2% gum acacia solution. Group 2: diabetic control rats administered 2% gum acacia solution. Group 3: diabetic animals were administered glibenclamide [600 μg/kg; per os (p.o.)]. Groups 4 and 5: diabetic animal were administered orally 200 and 400 mg/kg p.o. ethanolic extract of *Psoralea corylifolia* seeds respectively daily for 28 days.

Sample Collection

Blood Sample: The 24 hr fasted animals were sacrificed by cervical decapitation on 29th day of treatment. Trunk blood was collected in heparinized tubes and the plasma was obtained by centrifugation at 5000 rpm for 5 min. It was used for the determination of biochemical parameters like plasma glucose, plasma insulin level, cholesterol, malondialdehyde (MDA), reduced glutathione (GSH), *etc*. While whole blood was used for glycosylated hemoglobin.

Collection of Organs —— The rats were euthanized by using the overdose of intraperitoneal anesthesia, and tissue sample were taken for assessment of biochemical parameters.

Estimation of Plasma Glucose and Cholesterol —— Plasma cholesterol and glucose level were measured by commercial supplied biological kit Erba Glucose Kit (GOD-POD Method) and Erba Cholesterol Kit (CHOD-PAP Method) respectively using Chem 5 Plus-V$_2$ Auto-analyser (Erba, Mannheim, Germany) in plasma sample prepared as above. Glucose and cholesterol values were calculated as mg/dl blood sample.

Estimation of Glycosylated Hemoglobin (Hb1Ac) —— Glycosylated hemoglobin was

measured using commercial supplied biological kit (Erba Diagnostic) in plasma sample prepared as above using Chem 5 Plus-V$_2$ Auto-analyser (Erba). Values are expressed as the percent of total hemoglobin.

Estimation of MDA Level —— MDA, an index of free radical generation/lipid peroxidation, was determined as described by Ohkawa *et al.* 1979.[19] Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium lauryl sulphate, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid added to 0.2 ml of blood plasma. The mixture was made up to 4.0 ml with distilled water and heated at 95°C for 60 min. After cooling the contents under running tap water, 5.0 ml of n-butanol and pyridine (15 : 1 v/v) and 1.0 ml of distilled water was added. The contents were centrifuged at about 3000 rpm for 10 min. The organic layer was separated out and its absorbance was measured at 532 nm using double beam UV-Visible spectrophotometer (Systronics 2203, Bangalore, India) against a blank. MDA values are calculated using the extinction coefficient of MDA-thiobarbituric acid complex 1.56×10^5 l/mol $\times$ cm and expressed as nmol/ml.[19]

Estimation of Plasma Reduced Glutathione Level —— The tissue sample (liver 200 mg) was homogenized in 8.0 ml of 0.02 M EDTA in an ice bath. The homogenates were kept in the ice bath until used. Aliquots of 5.0 ml of the homogenates were mixed in 15.0 ml test tubes with 4.0 ml distilled water and 1.0 ml of 50% trichloroacetic acid (TCA). The tubes were centrifuged for 15 min at approximately $3000 \times g$, 2.0 ml of supernatant was mixed with 4.0 ml of 0.4 M Tris buffer pH 8.9, 0.1 ml Ellman's reagent DTNB added and the sample shaken. The absorbance was read within 5 min of the addition of DTNB at 412 nm against a reagent blank with no homogenate. Results were expressed as μmol GSH/g tissue.[20]

Estimation of Liver Glycogen Content —— Liver glycogen estimation was done by the method as described by Seifter *et al.*[21] Immediately after excision from the animal, 1 g of the liver was dropped into a previously weighed test tube containing 3 ml of 30% potassium hydroxide solution. The weight of the liver sample was determined. The tissue was then digested by heating the tube for 20 min in boiling water bath, and following this the digest was cooled, transferred quantitatively to a 50 ml volumetric flask, and diluted to the mark with water. The contents of the flask were then thoroughly mixed

and a measured portion was then further diluted with water in a second volumetric flask so as to yield a solution of glycogen of 3–30 μg/ml. Five ml aliquots of the final dilution were then pipette into Evelyn tube and the determination with anthrone was carried out. The amount of glycogen in the aliquot used was then calculated using the following equation:

$$\mu g \text{ of glycogen in aliquot} = 100 \, U/1.11S$$

U is the optical density of unknown solution. S is the optical density of the 100 μg glucose and 1.11 is the factor determined by Morris in 1948 for the conversion of the glucose to the glycogen.[21]

Serum Insulin Assay by ELISA Kit —— Serum insulin level was measured by an enzyme-linked immunosorbent assay (ELISA) procedure using Mercodia rat insulin ELISA kit. Briefly, the solid phase two-site enzyme immunoassay is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants 35 (epitopes) on the insulin molecule. During incubation, insulin in the sample reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to the micro titration well. After washing three times, unbound enzyme labeled antibody was removed. The bound conjugated insulin was detected by reacting with 3,3′,5,5′-tetramethylbenzidine. The reaction was stopped by adding acid to give a colorimetric end-point and optical density was measured with a micro plate auto reader (Bio-tek Instrument Inc., Winooski, VT, U.S.A.) at a wavelength of 450 nm. The serum insulin was expressed as μg/l.

Statistical Analysis —— The data for various biochemical parameters were evaluated by use of one-way analysis of variance (ANOVA), followed by Dunnett's t-test using the software Sigma-Stat 3.5. In all the tests, the criterion for statistical significance was $p < 0.05$.

RESULTS

Oral Glucose Tolerance Test

The effect of *Psoralea corylifolia* extract on plasma glucose level after glucose loading at 2 g/kg body weight (b.wt.) orally to the STZ diabetic rats is expressed in the Fig. 1. The blood glucose level rises to a maximum in 30 min after glucose loading. The extract treated groups at both 200 and 400 mg/kg b.wt. showed a significant increase in
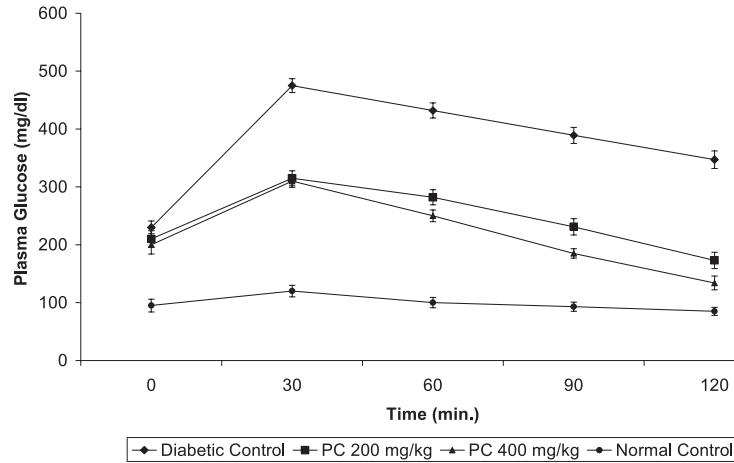
Fig. 1. Effect of *Psoralea corylifolia* on Oral Glucose Tolerance Test

Values are represented as mean ± S.E.M., *n* = 6 in each group. One way ANOVA followed by Dunnett's test; PC: *Psoralea corylifolia*.
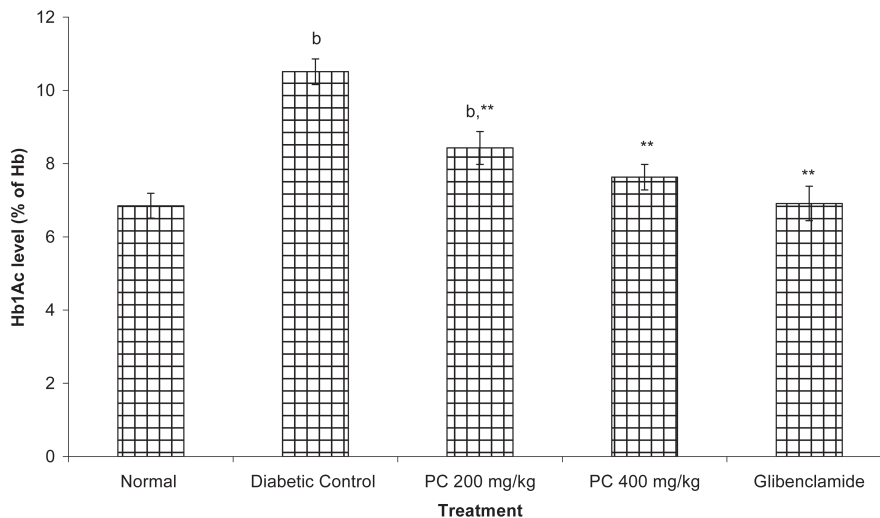

Fig. 2. Effect of *Psoralea corylifolia* on Glycosylated Hemoglobin HbA1c

Values are presented as mean ± S.E.M.; one way ANOVA followed by Dunnett's test; [b] $p < 0.01$ when compared to normal rats values, [**] $p < 0.01$ when compared to diabetic control group. PC: *Psoralea corylifolia*.

rate of clearance of glucose as compared to untreated group. The extract treated group showed a marked fall in glucose level in 30–90 min interval. After 120 min the serum glucose level returns to normal value.

Effect of *Psoralea corylifolia* on Glycosylated Hemoglobin HbA1c

The effect of ethanolic seed extract of *Psoralea corylifolia* on glycosylated hemoglobin HbA1c in STZ diabetic rats is shown in the Fig. 2. The level of glycosylated hemoglobin significantly increased in diabetic rats as compared to normal control group. The diabetic rats when treated with *Psoralea corylifolia* extract at a dose of 200 mg/kg orally for

28 days showed a decreased level of glycosylated Hb as compared to untreated diabetic group. Higher dose showed more fall in glycosylated hemoglobin level.

Effect of *Psoralea corylifolia* on Liver Glycogen

The liver glycogen content in diabetic rats decreased sharply when compared to normal control animal as shown in the Fig. 3. After chronic administration of *Psoralea corylifolia* to diabetic rats for 28 days there was a significant increase in the liver glycogen content $p < 0.05$ (44 ± 4.23 mg/g) for 200 mg/kg b.wt. and $p < 0.01$ (53.69 mg/g) for 400 mg/kg b.wt. p.o. as compared to diabetic control group.
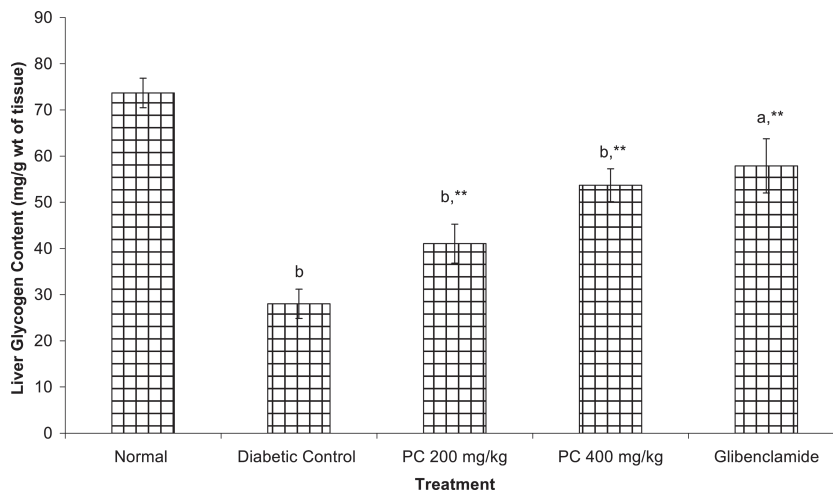
Fig. 3. Effect of *Psoralea corylifolia* on Liver Glycogen
Values are presented as mean ± S.E.M.; one way ANOVA followed by Dunnett's test; [a] $p < 0.05$, [b] $p < 0.01$ when compared to normal rats values, [**] $p < 0.01$ when compared to diabetic control group. PC: *Psoralea corylifolia*.

Fig. 4. Effect of *Psoralea corylifolia* on Plasma Cholesterol
Values are presented as mean ± S.E.M.; one way ANOVA followed by Dunnett's test; [a] $p < 0.05$, [b] $p < 0.01$ when compared to normal rats values, [**] $p < 0.01$ when compared to diabetic control group. PC: *Psoralea corylifolia*.
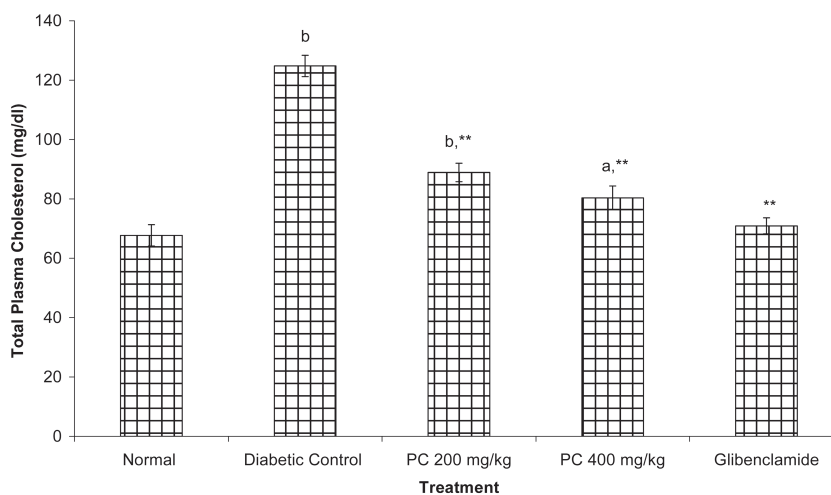
Effect of *Psoralea corylifolia* on Plasma Cholesterol

The data presented in Fig. 4 indicated the effect of *Psoralea corylifolia* seeds extract on total plasma cholesterol. Total plasma cholesterol was observed significantly higher (124.78 ± 3.58 mg/dl; $p < 0.01$) in diabetic rats compared to normal rats (67.69 ± 3.60 mg/dl). The chronic administration of the control drugs significantly lowered the total plasma cholesterol concentration. The administration of *Psoralea corylifolia* with both 200 and 400 mg/kg, p.o. showed significant reduction to high extent in total plasma cholesterol level to 88.89 ± 3.1 mg/dl ($p < 0.01$) and 80.32 ± 4.01 mg/dl ($p < 0.01$) from 124.78 ± 3.58 mg/dl.

Effect of *Psoralea corylifolia* MDA

The data depicted in Fig. 5 indicates the effect of *Psoralea corylifolia* on plasma malondialdehyde level. Plasma MDA level was found to be significantly higher in n-STZ diabetic rats compared to normal rats. The *Psoralea corylifolia* seeds extract 200 and 400 mg/kg b.wt. p.o. significantly reduced the level of MDA in diabetic rats from 4.8 ± 0.43 to 3.1 ± 0.23 nmol/dl ($p < 0.01$) and 2.4 ± 0.33 nmol/dl respectively.
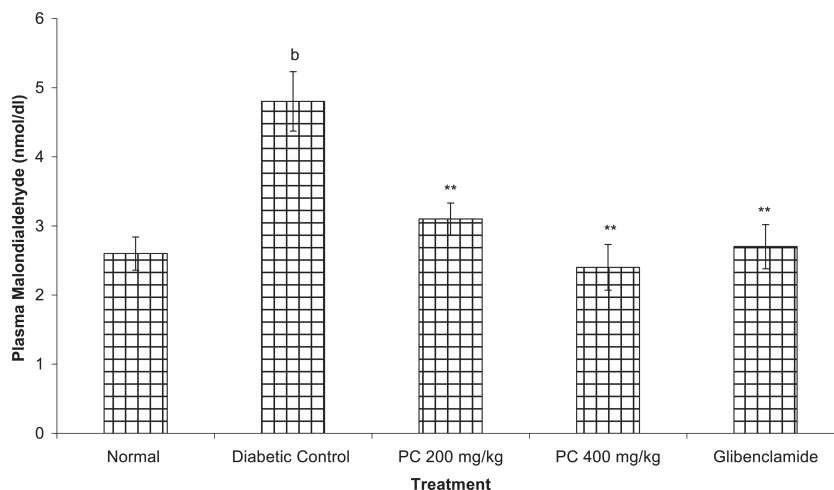
Fig. 5. Effect of *Psoralea corylifolia* on Plasma Malondialdehyde Level (MDA)
Values are presented as mean ± S.E.M.; one way ANOVA followed by Dunnett's test; [b] $p < 0.01$ when compared to normal rats values, [**] $p < 0.01$ when compared to diabetic control group. PC: *Psoralea corylifolia*.
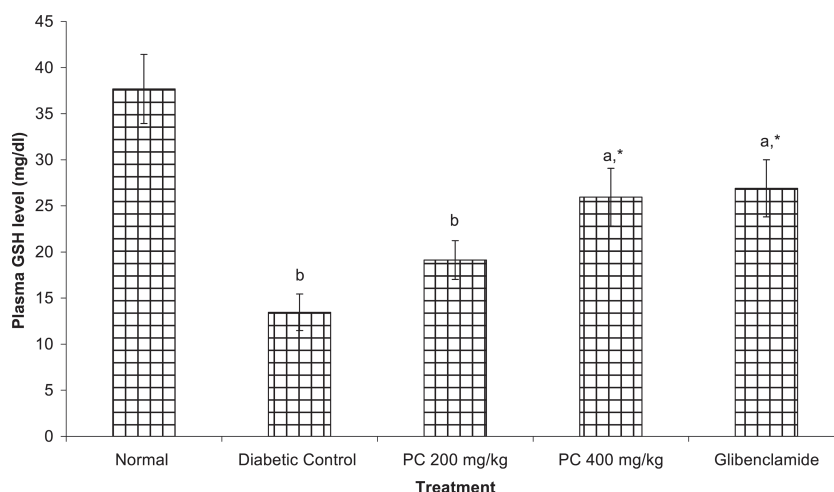

Fig. 6. Effect of *Psoralea corylifolia* on Plasma Reduced Glutathione (GSH)
Values are presented as mean ± S.E.M.; one way ANOVA followed by Dunnett's test; [a] $p < 0.05$; [b] $p < 0.01$ when compared to normal rats values, [*] $p < 0.05$ when compared to diabetic control group. PC: *Psoralea corylifolia.*

Effect of *Psoralea corylifolia* on GSH

The effect of *Psoralea corylifolia* seeds extract on glutathione is shown in Fig. 6 which indicates its antioxidant potential. Plasma GSH level was found to be significantly lowers in STZ diabetic rats as compared to normal rats. The chronic administration of *Psoralea corylifolia* 200 and 400 mg/kg b.wt. significantly increased the level of glutathione in diabetic rats from 13.45 ± 1.99 to 19.12 ± 2.10 mg/dl and 25.94 ± 3.13 mg/dl ($p < 0.05$) correspondingly.

Effect of *Psoralea corylifolia* on Acute Antihyperglycemia

Administration of ethanol extract of *Psoralea*

corylifolia at a dose 200 mg/kg b.wt. p.o. to STZ diabetic rats showed reduction in blood glucose level (BGL) to 5.27% ($p < 0.05$) at 2 hr followed by 15.83 ($p < 0.01$), 30.36 ($p < 0.01$) and 2.5% ($p < 0.01$) at 4, 6 and 24 hr respectively when compared to 0 hr. Administration of *Psoralea corylifolia* extract in a dose of 400 mg/kg b.wt. p.o. showed significant glucose lowering effect at 2, 4 and 6 hr with a decrease of 10.35, 21.66, and 35.56% in plasma glucose level respectively. The higher dose of *Psoralea corylifolia* had more blood glucose lowering effect as compared to lower dose as shown in Table 1.

Table 1. Effect of *Psoralea corylifolia* on STZ Induced Diabetic Rats in Acute Anti-hyperglycemic Model

Treatment	Blood glucose levels (mg/dl) at the respective time				
(Dose)	0 hr	2 hr	4 hr	6 hr	24 hr
Diabetic control	336.16 ± 3.2	339.05 ± 4.78	331.88 ± 3.02	342.79 ± 5.08	330.66 ± 4.7
PC (200 mg/kg)	341.53 ± 4.9	$323.35 \pm 3.6^*$	$287.31 \pm 3.6^{**}$	$237.82 \pm 5.4^{**}$	332.50 ± 4.6
PC (400 mg/kg)	346.80 ± 5.57	$311.12 \pm 2.78^*$	$271.66 \pm 2.23^{**}$	$223.47 \pm 5.7^{**}$	329.18 ± 6.21
Glibenclamide (600 µg/kg)	332.85 ± 3.6	$302.58 \pm 4.58^{**}$	$262.82 \pm 2.08^{**}$	$219.04 \pm 4.37^{**}$	326.27 ± 3.45

Values are presented as mean ± S.E.M.; $n = 6$ in each group. One way ANOVA followed by Dunnett's test, $^*p < 0.05$; $^{**}p < 0.01$; PC: *Psoralea corylifolia*, Glibenclamide (600 µg/kg b.wt. p.o.).

Table 2. Effect of *Psoralea corylifolia* on STZ Induced Diabetic Rats on Chronic Anti-hyperglycemic Model

Treatment	Blood glucose levels (mg/dl) at the respective day				
(Dose)	0th	7th	14th	21st	28th
Diabetic control	331.40 ± 5.52	349.10 ± 6.25	363.43 ± 6.31	380.07 ± 4.01	389.82 ± 6.6
PC (200 mg/kg)	337.31 ± 4.79	$226.05 \pm 4.36^*$	$208.40 \pm 7.03^{**}$	$182.32 \pm 4.8^{**}$	$164.11 \pm 5.29^{**}$
PC (400 mg/kg)	329.53 ± 3.90	$214.33 \pm 4.10^{**}$	$201.26 \pm 6.67^{**}$	$178.61 \pm 5.20^{**}$	$159.78 \pm 6.20^{**}$
Glibenclamide (600 µg/kg)	342.84 ± 5.27	$210.59 \pm 5.43^{**}$	$186.12 \pm 5.81^{**}$	$153.74 \pm 5.31^{**}$	$144.45 \pm 3.9^{**}$

Values are presented as mean ± S.E.M.; $n = 6$ in each group. One way ANOVA followed by Dunnett's test, $^*p < 0.05$; $^{**}p < 0.01$; PC: *Psoralea corylifolia*, Glibenclamide (600 µg/kg b.wt. p.o.).

Table 3. Effect of *Psoralea corylifolia* on Body Weight and Plasma Insulin Level

Treatment	Initial body	Final body	Plasma insulin microunit/ml
(Dose)	weight (g)	weight (g)	(µU/ml)
Normal	216 ± 5.16	223 ± 2.13	14.13 ± 0.93
Diabetic control	212 ± 4.83	193 ± 4.28	$4.32 \pm 0.52^{a)}$
PC (200 mg/kg)	210 ± 5.41	$219 \pm 5.69^*$	$7.93 \pm 1.08^{a),*}$
PC (400 mg/kg)	213 ± 7.74	$228 \pm 4.48^{**}$	$9.6 \pm 1.02^{a),**}$
Glibenclamide (600 µg/kg)	217 ± 3.19	$238 \pm 3.54^{**}$	$12.44 \pm 1.01^{**}$

Values are presented as mean ± S.E.M.; one way ANOVA followed by Dunnett's test; a) $p < 0.01$ when compared to normal rats values, $^*p < 0.05$, $^{**}p < 0.01$ when compared to diabetic control group, PC: *Psoralea corylifolia*.

Effect of *Psoralea corylifolia* on Chronic Hyperglycemia

In the chronic study of *Psoralea corylifolia* 200 mg/kg b.wt. p.o. to STZ diabetic rats for 28 days showed a fall in serum glucose level 32.98 ($p < 0.05$), 38.21 ($p < 0.01$), 45.94 ($p < 0.01$), 51.37% ($p < 0.01$) at 7th, 14th, 21st and 28th day when compared to 0 day value. At 400 mg/kg b.wt. p.o. there was a significant fall in plasma glucose level from 34.95% ($p < 0.01$) at 7th day to 45.79% ($p < 0.01$) at 21st day, at 28th day to 51.51% as shown in Table 2.

Effect of *Psoralea corylifolia* on Body Weight and Plasma Insulin

The body weight of diabetic rats became less as compared to the initial weight as shown in the Table 3. In case of *Psoralea corylifolia* treated groups both at 200 and 400 mg/kg b.wt. there was a significant gain in the body weight of animals when compared to the diabetic control group. The weight gain in the extract treated rats was comparable to the glibenclamide treated rats.

The Table 3 also gives the levels of plasma insulin in the control and experimental groups of rats. Diabetic rats showed a significant decrease in plasma insulin compared to diabetic rats. Following dose of oral administration of *Psoralea corylifolia* extract and glibenclamide, plasma insulin levels increased significantly as compared to diabetic rats.

DISCUSSION

Experimental diabetes mellitus has been induced in laboratory animals by several methods. Experimental diabetes has been used to analyze the biochemical, hormonal and morphological changes that take place during the induction of a diabetes state and after it has taken place. This method has great advantages but it has to be specify that non of animal models with induced diabetes similar to the human type-2 diabetic mellitus, though they design models to investigate the pathogenic mechanism that lead to hyperglycemia and consequences.[22]

Masiello *et al.* described a new experimental diabetic model in adult rats by administering STZ and partially protected it with a suitable dose of nicotinamide. This syndrome shares a number of features with human type-2 diabetes, and is characterized by moderate stable hyperglycemia, glucose intolerance, etc., *in vivo* and *in vitro*.[18] Hyperglycemia causes oxidative damage by the generation of reactive oxygen species[23] and results in the development of diabetic complications, *i.e.* cardiovascular, gastrointestinal, nervous, vas deferens, kidney and urinary bladder dysfunctions.[24—26] The increase in oxygen free radicals in diabetes could be primarily due to the increase in blood glucose level and secondarily due to the effect of diabetogenic agent streptozotocin.[26]

Phytochemical investigation of *Psoralea corylifolia* shows the presence of flavanoids and terpenoids. It is well known that certain flavonoids has hypoglycemic activity[27, 28] and are also known for their ability of beta cell regeneration of pancreas.[29] Thus, the significant antidiabetic effect of alcoholic extract of *Psoralea corylifolia* may be due to the presence of more than one antihyperglycemic principle and their synergic property.

The present study was undertaken with the objective of exploring the antidiabetic potential of *Psoralea corylifolia* in STZ-nicotinamide induced type-2 diabetic rats. In acute antihyperglycemic model Glibenclamide was taken as a reference drug and extract of the plant shown significant reduction in blood glucose level in the dose dependent manner. Glibenclamide is an anti-diabetic drug in a class of medications known as sulfonylureas, closely related to sulfa drugs. It is used in the treatment of type-2 diabetes. As of 2007, it is one of only two oral anti-diabetics in the World Health Organization model list of essential medicines (the other being metformin). As of 2003, in U.S.A., it was the most popular sulfonylurea. The drug works by inhibiting adenosine tri-phosphate (ATP)-sensitive potassium channels in pancreatic beta cells. This inhibition causes cell membrane depolarization, which causes voltage-dependent calcium channels to open, which causes an increase in intracellular calcium in the beta cell, which stimulates insulin release. Thus the drug acts as positive control in type-2 diabetes.

The reduction in glucose level is significant ($p < 0.01$) in treated animals at 2, 4 and 6 hr after drug administration. The glucose lowering activity of *Psoralea corylifolia* may be related to both pancreatic (enhancement of insulin secretion) and extra pancreatic (peripheral utilization of glucose) mechanism. The prolonged treatment of *Psoralea corylifolia* extract for 28 days on STZ rats not only produced consistent reduction in blood glucose levels but also improved the glucose tolerance in STZ rats. Both dose of the extract have shown significant ($p < 0.01$) decrease in blood glucose during the 7th day of the treatment and level consistently decrease for further 21 days. The higher dose (400 mg/kg b.wt. p.o.) of the extract have more marked reduction in blood glucose 34.95% ($p < 0.01$) at 7th day than lower dose (200 mg/kg b.wt. p.o.), 32.98% ($p < 0.05$).

In STZ induced diabetes, the loss of body weight caused by increase in muscle wasting[30] and catabolism of fat and proteins. Due to insulin deficiency protein content is decreased in muscular tissue by proteolysis.[31] A decrease in body weight was registered in case of STZ diabetic control group rats while in treated group the weight loss was reversed. The body weight of diabetic rats at doses 200 and 400 mg/kg b.wt. were observed to be increased by 11.40 and 15.16% respectively.

Further, the antihyperglycemic activity of *Psoralea corylifolia* was associated with an increase in plasma insulin level, suggesting insulin secreting activity of the seed extract which stimulates insulin secretion from the remnant β-cells or from regenerate β-cells. There are number of plants have been reported to exert there hypoglycemic activity through insulin release stimulatory effect.[32, 33]

There is increase in the level of glycosylated hemoglobin (HbA1c) in the diabetic control group of rats due to the presence of large amount of blood glucose. Large amount of glucose present in diabetic condition react with hemoglobin to form glycosylated hemoglobin.[34—36] Oxidative stress in-

creased due to the activation of transcription factors, advanced glycated end products (AGEs), and protein kinase C. If the condition of diabetes is persistent for long time, the glycosylated hemoglobin has been found to be increased.[36,37] The levels of HbA1c decreased from diabetic control group $10.51 \pm 0.27\%$ to $8.43 \pm 0.29\%$ and $7.63 \pm 0.35\%$ at dose of *Psoralea corylifolia* 200 and 400 mg/kg b.wt. respectively which is approximately near to normal ($6.85 \pm 0.34\%$).

In present study, we observed elevated levels of plasma lipids such as cholesterol in diabetic rats. The levels of increased serum lipids in diabetes represent a risk factor for coronary heart disease.[38] In the present study, marked increase in total cholesterol level (124.78 ± 3.58 mg/dl) was observed in diabetic rats compared to normal rats (67.69 ± 3.6 mg/dl). Insulin increases uptake of fatty acids into adipose tissue and increases triglyceride synthesis. Moreover, insulin inhibits lipolysis. In case of insulin deficiency, lipolysis is not inhibited and we have increased lipolysis which finally leads to hyperlipidemia.

The conversion of glucose to glycogen in the liver cells is dependent on the extracellular glucose concentration and on the availability of insulin which stimulates glycogen synthesis over a wide range of glucose concentration.[39] Glycogen store and synthesis in rat liver and skeletal muscle is impaired in diabetes[40] due to the reduced activity of glycogen synthase. Oral administration of *Psoralea corylifolia* 200 mg/kg b.wt. significantly increases hepatic glycogen levels in STZ diabetic rats from 28.01 ± 3.17 to 44.04 ± 4.23 mg/g possibly because of the reactivation of the glycogen synthase system as a result of increased insulin secretion. Higher dose 400 mg/kg b.wt. showed an increase up to 53.69 ± 3.55 mg/g in the glycogen level.

The oxidative stress plays a major role in the development and progression of both type-1 and type-2 diabetes mellitus and also contributes in their adverse effects. Many researches proved the role of antioxidant compound in both protection and therapy of diabetes mellitus. There is an evidence that glycosylation of various protein may itself induce the generation of oxygen-derived free radicals in diabetic condition.[41,42] Hyperglycemia results in the generation of free radical which can exhaust antioxidant defense thereby leading to the disruption of cellular function, oxidative damage to membrane and enhance the susceptibility to lipid peroxidation[43] and diffuse from the site of tissue damage

which is measured by malondialdehyde level. A significant increase in serum malondialdehyde level was observed in diabetic rats (4.8 ± 0.43 nmol/ml) compared to normal rats (2.6 ± 0.24 nmol/ml). *Psoralea corylifolia* extract significantly ($p < 0.01$) decreased lipid peroxidation in diabetic rats at both 200 and 400 mg/kg oral doses. The extract at 400 mg/kg p.o. dose showed marked reduction in the serum MDA level (2.4 ± 0.33 nmol/ml). It showed that marked reduction in serum MDA level is a result of both direct antioxidant action and glycaemic control.

The level of glutathione (GSH) in diabetic rats (13.45 ± 1.99 mg/dl) is lower than normal rats (37.68 ± 3.78 mg/dl) which indicate altered antioxidant system during diabetes.[44] Reduced glutathione plays major role in regulation of cellular redox state. The imbalance in reduced glutathione to oxidized glutathione is a indicator of cellular oxidative stress.[45] Decreased level of glutathione in serum of STZ diabetic rats was partly due to its utilization by the tissue to compromise the deleterious effect of lipid peroxidation.[46,47] *Psoralea corylifolia* extract (400 mg/kg p.o.) increased the level of GSH to (25.94 ± 3.13 mg/dl) in STZ diabetic rats, which is an indication of its antioxidant properties. In the study, reduced glycosylated hemoglobin level and improved glutathione level in extract treated rats was observed and gives a negative correlation between GSH and HbA1c in diabetic animal as reported by Giugaliano *et al.*, 1996, which confirmed the link between hyperglycemia and GSH depletion. In hyperglycemic condition, glucose is used in polyol pathway,[48] which consumes NADPH necessary for GSH regeneration by the GSH-reductase enzyme. Hyperglycemia is therefore indirectly the cause of GSH depletion. As GSH is an important antioxidant molecule, its depletion leads to the increased of oxidative stress.

In conclusion, the present study shows that the ethanolic extract of *Psoralea corylifolia* seeds has potential anti-hyperglycemic and antioxidant effect in STZ-induced diabetic rats. The mechanism of action of anti-hyperglycemic action may involve improved insulin secretion and peripheral glucose utilization, *etc*. Further, studies are in progress at molecular level to explain more about the mechanism of anti-diabetic activity of *Psoralea corylifolia* and active constituent which is responsible for its anti-diabetic effect.

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